# Determination of Catalase Activity and its Inhibition by a Simple Manometric Method

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## Introduction

There has been a considerable amount of controversy about the use of manometric methods to measure catalase activity. As Maehly and Chance point out in their excellent review,[1] the advantages of these methods is ". . . that they can be used for any kind of biological material, and purification of the enzyme is not required. The assay is independent of small amounts of peroxidase activity. It is fairly simple to perform, it is rapid and it can be adapted to continuous reading of the reaction". A variety of drawbacks are also listed by the same authors, viz, the inactivation of the enzyme under the experimental conditions and the time lag before a constant rate of oxygen evolution is reached.

The objections raised by numerous researchers, and the emergence of accurate titrimetric methods, has led to the progressive abandonment of manometric assays for catalase activity. We are nevertheless convinced that the catalase activity assessment by a manometric technique is still useful as a teaching experiment. Indeed, an appropriate experimental design may be a good way of familiarizing students with manometric techniques. Furthermore, catalase is an enzyme which is well fitted for lab teaching, as its natural sources are abundant and cheap (eg blood lysates or liver homogenates). Last but not least, the materials needed are so inexpensive that the experiments can be performed even in high schools where resources are scarce.

### Materials

Hydrogen peroxide was titrated by the iodometric method proposed by Chance and Maehly,[2] and diluted with 0.03 M phosphate buffer, pH 7.0.[3] Catalase in granulated form was purchased from Sigma with indicated activity 2600 units/mg solid. This is not a pure preparation and according to Sigma, one unit catalyses the decomposition of 1.0 μmol of hydrogen peroxide per minute at pH 7 and 25°C when the peroxide concentration lies between 10.3 and 9.2 mM.

The other source of catalase used was human blood: 10 μl blood collected from a volunteer was immediately dropped into a conical flask containing 10 ml of cold phosphate buffer and surrounded by crushed ice. Sodium azide, 0.015 M was prepared in the phosphate buffer.

*Water manometer* A glass, 5 mm diameter U-tube was used for the construction of a constant pressure manometer. The tube, length about 100 cm, was fastened to a wooden block coated with millimetric graph paper (see Fig 1). The tube was partially filled with water coloured with safranin to improve scale readability. Silicone tubing, about 100 cm of 5 mm internal diameter, is also needed, as well as two clamps, one two-holed rubber stopper, a tap funnel, a magnetic bar and stirrer; a water bath, a 500 ml Buchner type filter flask and a stop-watch.

### Experimental

The hydrogen peroxide solution (50 ml) is placed in the filter flask, which was immediately plugged, and stirred (at about 120 rpm) for 10 min to allow thermal equilibration. This stage also allows for the release of oxygen in equilibrium with the peroxide solution. In this stage the two clamps are open.

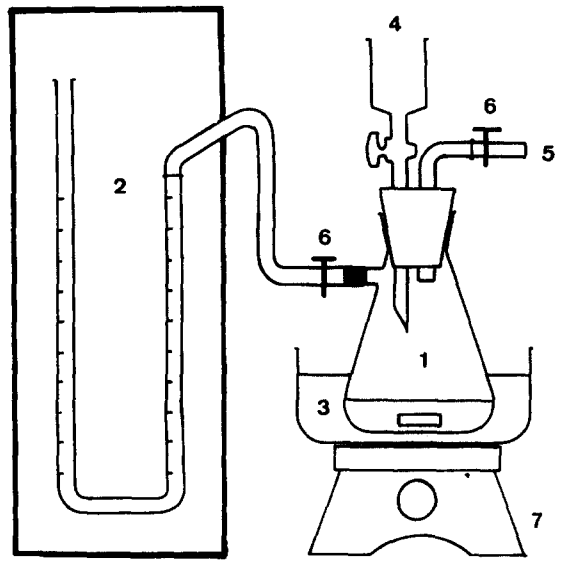


*Figure 1 Experimental set-up: (1) Buchner flask; (2) water manometer; (3) water bath; (4) tap funnel; (5) gas vent; (6) clamps; (7) magnetic stirrer*

Meanwhile, 10 ml of catalase solution are placed in the tap funnel. When 10 minutes of stirring have passed, the catalase solution is poured into the flask. After 30 seconds, the gas vent to the atmosphere is closed and the stop-watch started. Manometer readings (in mm of water) are taken at 30 s intervals for a total time of about 15 min. A plot of pressure (in mm of water) *versus* time (in min) enables the initial rates to be determined (see Fig 2). The values obtained may easily be converted to mol peroxide consumed per min. Now the equation for the decomposition of hydrogen peroxide is

$$H_2O_2 \rightarrow H_2O + 1/2\ O_2 \qquad (1)$$

which means that for every mole of evolved oxygen two moles of peroxide are consumed. Thus, if $n_o$ is the initial number of moles, $n$, the number of moles of peroxide present at any time is given by

$$n = n_o - N_o \qquad (2)$$

where $N_O$ is the number of moles of oxygen evolved. Using the ideal gas equation, we can write

$$V_L S = V_L S_o - (2PV_G/RT) \qquad (3)$$

where $V_L$ and $V_G$ are the liquid and gas volumes respectively, and $S_o$ and $S$ are the substrate (ie peroxide) concentration at

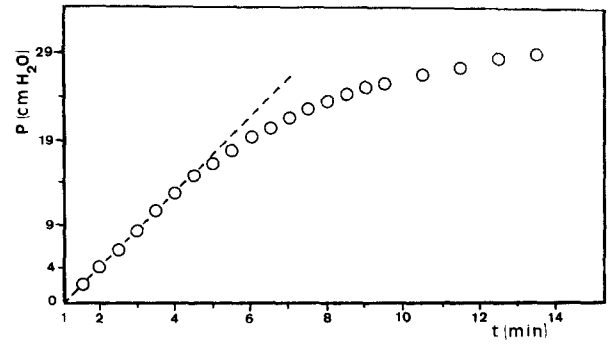


*Figure 2 Oxygen evolution with time*

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zero and at any given time, respectively. Rearranging eqn 3 we obtain

$$S = S_o - (2P/RT)\,(V_G/V_L) \quad (4)$$

The peroxide consumption rate can therefore be obtained from eqn 4 as follows

$$v = -dS/dt = (2P/RT)(V_G/V_L)(dP/dt) \quad (5)$$

In order to obtain $v$ from eqn 5, we need to know the values of $V_G$ and $V_L$. The liquid volume is 60 ml. The volume occupied by the gas at time zero may be determined by measuring the water volume needed to completely fill the void volume in the filter flask and tubes. In our case the total gas volume was 555 ml.

*First Laboratory Session* The first laboratory period (about 3 h) is devoted to the determination of the kinetic parameters. Table 1 shows the initial substrate concentrations and the values calculated for the initial rates. A Lineweaver–Burk plot may be obtained (see Fig 4) and from this plot, the Michaelis constant and $V_{max}$ may be obtained. The data obtained (Table 2) are in quite close agreement with those reported in the literature.[4]

*Table 1 Initial rate of $O_2$ release ($v_o$) for several $H_2O_2$ concentrations ($S_o$)*

| $S_o \times 10^3$ (mol $l^{-1}$) | $v_o \times 10^5$ (mol $l^{-1}$ $s^{-1}$) |
|---|---|
| 14.1 | 44.5 |
| 18.8 | 44.9 |
| 23.4 | 65.3 |
| 28.1 | 66.4 |
| 32.8 | 70.2 |
| 37.5 | 82.3 |

*Table 2 Calculated kinetic parameters for catalase*

| |
|---|
| $v_{max} = 1.45 \times 10^{-3}$ mol $l^{-1}$ $s^{-1}$ |
| $K_m = 3.16 \times 10^{-2}$ M |

*Second Laboratory Session* During the second laboratory period the students investigate the effects of an inhibitor (sodium azide[5]) and of temperature. As will be seen (Fig 3), the value obtained for the optimum temperature was remarkably close to values reported in the literature.[6] It is necessary to take into account the thermal decomposition of hydrogen peroxide: for each working temperature a run without catalase is also carried out. However, oscillations in the readings were found above 60°C, and therefore, this was the upper temperature used. Activation energy calculations may be done from these results.

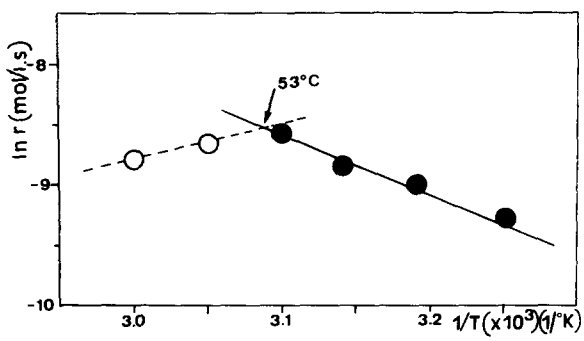


*Figure 3 Arrhenius plot for determination of optimal temperature: r = rate of oxygen evolution (mol $l^{-1}$ $s^{-1}$); T = absolute temperature (°K)*

The inhibition effect of sodium azide was also assessed. Fig 4 shows the Lineweaver–Burk plots obtained with 0.015 M of sodium azide and in the absence of inhibitor. In spite of the uncertainty associated with manometric techniques, the convergence of the two lines seems to indicate that the inhibition is probably of the noncompetitive type. However, the errors associated with this method cannot exclude other inhibition types, such as a mixed inhibition.

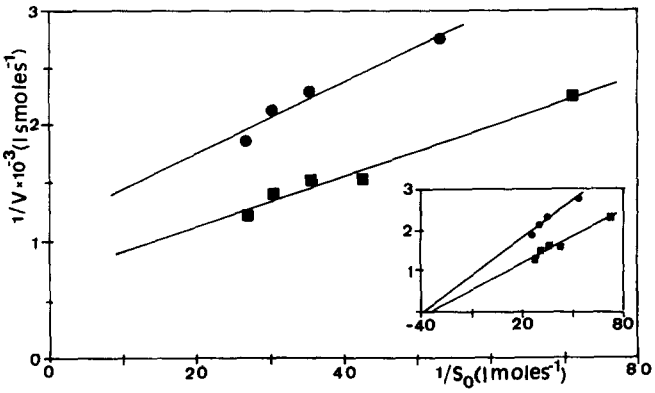


*Figure 4 Lineweaver–Burk plot for the determination of the kinetic parameters of catalase: ■ — without sodium azide, ● — with 0.015 M sodium azide*

## Conclusions

The experiments outlined here are effective, rapid and quite inexpensive and the whole practical class allows students to deal with concepts such as inhibition and energy of activation. Other kinds of studies may also easily be implemented. For example, the effect of pH or the combined effect of pH and temperature may be studied by repeating the experiments with a set of appropriate buffer solutions.

Slight variations in atmospheric pressure and in the temperature, and a slow but significant decomposition of hydrogen peroxide, especially at higher temperatures, are unavoidable. The students should therefore be aware that this method is not suitable for rigorous kinetic determinations.

## References

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